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Biocatalytic Cascade for the Synthesis of Enantiopure β-Azidoalcohols and β-Hydroxynitriles

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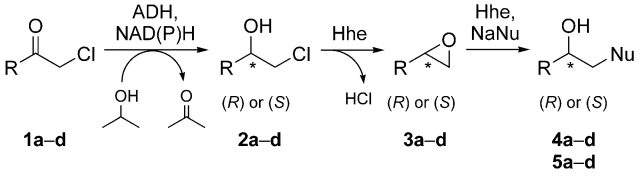
A three-step, two-enzyme, one-pot reaction sequence starting from prochiral α-chloroketones leading to enantiopure β-azidoalcohols and β-hydroxynitriles is described. Asymmetric bioreduction of α-chloroketones by hydrogen transfer catalysed by an alcohol dehydrogenase (ADH) established the stereogenic centre in the first step to furnish enantiopure chlorohydrin intermediates. Subsequent biocatalysed ring closure to the epoxide and nucleophilic ring opening with azide, N_3^-, or cyanide, CN^-, both catalysed by a nonselective halohydrin dehalogenase (Hhe) proceeded with full retention of configuration to give enantiopure β-azidoalcohols and β-hydroxynitriles, respectively. Both enantiomers of various optically pure β-azidoalcohols and β-hydroxynitriles were synthesised.

Introduction

Enantiopure β-azidoalcohols and β-hydroxynitriles are building blocks in the synthesis of pharmaceutical compounds such as β-blockers,[1] cholesterol lowering drugs[2] or antidepressants.[3] Methods for the asymmetric synthesis of these compounds include the stereoselective reduction of the corresponding ketones by applying metal[4] as well as enzyme catalysis,[5] kinetic resolution[6] or dynamic kinetic resolution[7] of the racemate by lipase-catalysed acylation and desymmetrisation of hydroxydinitriles.[8] The stereoselective ring opening of racemic epoxides is also widely used[9] but bears the disadvantage that the maximum theoretical yield of the desired enantiomer is limited to 50%.

We recently reported a biocatalytic cascade sequence starting from prochiral α-chloroketones leading to enantiopure epoxides.[10] Asymmetric bioreduction of the chloroketones catalysed by an alcohol dehydrogenase (ADH) followed by epoxide ring closure catalysed by a halohydrin dehalogenase (Hhe) furnished epoxides in excellent enantiomeric purity, although in rather limited yields due to the reversibility of the reaction.

In order to shift the equilibrium to the product side we now envisioned to extend the cascade by a nucleophilic ring-opening reaction to give β-substituted alcohols as the final products. As this reaction is also catalysed by halohydrin dehalogenases we expected a convenient three-step, two-enzyme, one-pot process (Scheme 1). Azide and cyanide were chosen as nucleophiles, because both are known to be accepted by the employed halohydrin dehalogenases,[11] and – more importantly – they react irreversibly to form the final products.



Scheme 1. Biocatalytic cascade sequence for the synthesis of enantiopure β-azidoalcohols and β-hydroxynitriles.

Results and Discussion

For the epoxide ring-closure and ring-opening reactions two different halohydrin dehalogenases were investigated: HheB from *Mycobacterium* sp. GP1,[12] as well as HheC from *Agrobacterium radiobacter* AD1.[11,12b] In order to test whether the conversion of β-halohydrins into the corresponding azidoalcohols and hydroxynitriles would give high conversion, *rac*-1-chloro-3-phenoxy-2-propanol (**2a**) was incubated with HheC in the presence of varied concentrations of azide and cyanide, respectively. With increasing concentration of the nucleophile, increasing conversion into **4a**/**5a** was achieved after 24 h, as higher concentration of the

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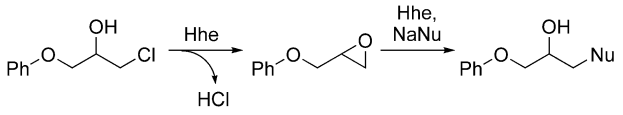
nucleophile corresponded to better availability and therefore a faster reaction (Table 1). Almost complete conversion into **4a/5a** was achieved in the most concentrated samples by employing HheC, which indicates that HheC exhibits a low stereoselectivity for halohydrin **2a**. This is in accordance with previous results that high enantioselectivities could only be obtained if 2,2-disubstituted oxiranes were used as substrates for the cyanide ring-opening reaction.[9e] In the absence of a nucleophile, only 29% of substrate **2a** was transformed into epoxide **3a** and 71% of substrate remained (Table 1, Entry 15). The ring opening with cyanide turned out to be slower than the one with azide, as high conversions of the hydroxynitrile could only be achieved with a large excess of NaCN, whereas 80 mM of NaN_3 gave almost 90% of the azidoalcohol (Table 1, Entries 4–7 vs. 11–14). However, as the buffer solutions with the highest CN^- concentration (320 and 640 mM) were not stable (precipitation of a brown solid[13] occurred within hours), a nucleophile concentration of 160 mM was used for all further experiments.

Table 1. Effect of the nucleophile concentration on the epoxide ring closure and ring opening catalysed by HheC.[a]



2a **3a** **4a/5a**

Entry	Nu	*c*(Nu) [mM]	**2a** [%][b]	**3a** [%][b]	**4a/5a** [%][b]
1	N_3^-	8	60	25	15
2	N_3^-	16	51	20	29
3	N_3^-	32	35	13	52
4	N_3^-	80	8	3	89
5	N_3^-	160	4	<0.1	96
6	N_3^-	320	2	<0.1	98
7	N_3^-	640	6	<0.1	94
8	CN^-	8	69	15	16
9	CN^-	16	64	9	27
10	CN^-	32	56	6	38
11	CN^-	80	52	5	43
12	CN^-	160	40	3	57
13	CN^-	320	24	1	75
14	CN^-	640	11	<0.1	89
15	none	–	71	29	<0.1

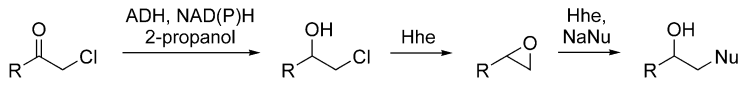
[a] Substrate **2a** (5 mg, 27 μmol) and HheC-preparation (15 μL, 65 U mL^{-1}) in 500 μL of Tris-SO_4 buffer (200 mM, pH 7.5) containing appropriate amounts of NaN_3 or NaCN were stirred (120 rpm) at 30 °C for 24 h. [b] Determined by GC analysis.

For the reduction of the chloroketones the two stereocomplementary alcohol dehydrogenases ADH-A from *Rhodococcus ruber* DSM 44541[14] and LBADH from *Lactobacillus brevis*[15] were used. Whereas the latter belongs to the short-chain dehydrogenase/reductase family and does not contain metal ions involved in the catalytic mechanism,[16] ADH-A contains a Zn^{2+} ion tightly bound in the active site.[17] Both nucleophiles, cyanide and azide, are known to complex Zn^{2+}, which might lead to inactivation of ADH-A in the cascade process. In order to test whether ADH-A stays active in the presence of CN^- and N_3^-, methyl-4-chloroacetoacetate (**1b**) was reduced with the use of ADH-A in the presence and absence of azide or cyanide anions (160 mM). The presence of azide did not have any effect on the reduction catalysed by ADH-A. However, ADH-A was not active in the presence of cyanide, as alcohol **2b** could not be detected. However, substrate **1b** was spontaneously transformed into methyl-4-cyanoacetoacetate, as proven by NMR spectroscopy as well as GC–MS. Thus, a nucleophilic substitution of chloride by cyanide had occurred. This reaction proceeded without involvement of the enzyme and has already been described in a patent for the corresponding bromo compound.[18] Because ADH-A was deactivated by CN^-, cyanide must not be added to the reaction mixture before all of the substrate has been reduced, that is, after a reaction time of 4 h. In contrast, it was shown that LBADH indeed stayed active in the presence of cyanide, but because the substitution of chloride by cyanide was spontaneous, the cyanoketone was also formed as a side product here. As only halohydrin was detected, but no hydroxynitrile, it was concluded that cyanoketone could not be reduced by these ADHs. In contrast, azidoketones were completely reduced by both ADHs to give the expected azidoalcohols.

With this background information we were able to couple the ADH- and Hhe-catalysed transformations and to establish the entire cascade process. Reactions employing cyanide as nucleophile were carried out in a stepwise fashion; thus, NaCN and the halohydrin dehalogenase were added after a reaction time of 4 h when the ketone was already reduced. For the cascade process involving azide, all reagents were mixed at the start of the reaction.

Employing 2-chloroacetophenone (**1c**) as substrate, another uncatalysed side reaction was observed. Uncatalysed ring opening of the epoxide intermediate (styrene oxide, **3c**) occurred at the benzylic position, especially in the presence of N_3^- as nucleophile. As a result of the high rate of this reaction, 2-azido-2-phenylethanol was formed as the main product instead of its desired regioisomer 2-azido-1-phenylethanol (**4c**). In the literature, the regio-unselective ring opening of racemic substituted styrene oxides with azide was circumvented by working at very low substrate and nucleophile concentrations or by adding NaN_3 continuously.[9c] Neither of these approaches seemed appropriate for a preparative transformation, so we tried to push back the uncatalysed reaction by applying azides with "softer" counterions (Cs^+, Bu_4N^+), but the effects were marginal. Apart from that, in all other cases the cascade afforded the desired β-substituted alcohols in good to excellent yields and perfect enantiomeric purity (Table 2). HheC reacted slightly faster with the *Prelog*-(*R*)-reduction products; therefore, it was employed in combination with ADH-A whereas HheB was used for the reaction sequences employing LBADH. Additionally, HheB was used in the cyanide cascade for substrate **1d** with ADH-A, as lower conversions were reached with the use of HheC (Table 2, Entry 15). In all cases measured, the optical purity of the obtained β-azidoalcohols and β-hydroxynitriles was perfect (>99% *ee*); thus, no trace of the second enantiomer could be detected.

Table 2. Three-step, two-enzyme cascade processes for the synthesis of β-azidoalcohols and β-hydroxynitriles.[a]



1 2 3 4/5

Entry	Substrate	Nu	ADH	Hhe	*t* [h]	Ketone **1** conc. [%][b]	Alcohol **2** conc. [%][b]	Epoxide **3** conc. [%][b]	Alcohol **4/5** conc. [%][b]	Alcohol **4/5** *ee* [%][c]
1	**1a**	N_3^-	ADH-A	HheC	24	<0.1	2	<0.1	98	>99 (*S*)
2	**1a**	N_3^-	LBADH	HheB	24	<0.1	20	<0.1	80	>99 (*R*)
3	**1b**	N_3^-	ADH-A	HheC	24	<0.1	5	<0.1	95	>99 (*R*)
4	**1b**	N_3^-	LBADH	HheB	24	<0.1	66	<0.1	34	>99 (*S*)
5	**1c**	N_3^-	ADH-A	HheC	24	<0.1	5	4	21[d]	n.d.[e]
6	**1c**	N_3^-	LBADH	HheB	24	<0.1	50	2	7[f]	n.d.[e]
7	**1d**	N_3^-	ADH-A	HheC	24	<0.1	<0.1	<0.1	>99	>99 (*R*)
8	**1d**	N_3^-	LBADH	HheB	24	<0.1	<0.1	<0.1	>99	>99 (*S*)
9	**1a**	CN^-	ADH-A	HheC	48	<0.1	7	1	92	>99 (*S*)
10	**1a**	CN^-	LBADH	HheB	24	<0.1	7	<0.1	93	>99 (*R*)
11	**1b**	CN^-	ADH-A	HheC	48	<0.1	34	<0.1	66	>99 (*S*)[i]
12	**1b**	CN^-	LBADH	HheB	24	<0.1	13	<0.1	87	>99 (*R*)[i]
13	**1c**	CN^-	ADH-A	HheC	48	<0.1	1	1	88[g]	>99 (*S*)
14	**1c**	CN^-	LBADH	HheB	24	<0.1	49	4	41[h]	>99 (*R*)
15	**1d**	CN^-	ADH-A	HheB	24	<0.1	4	14	82	>99 (*R*)
16	**1d**	CN^-	LBADH	HheB	24	<0.1	4	11	85	>99 (*S*)

[a] Azide reactions: 27–35 μmol substrate, 5 mg rehydrated cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A or 10 μL of LBADH stock solution (5 mg mL^{-1}, 1 mg mL^{-1} NADPH), 5 μL 2-propanol and 30 μL Hhe-preparation (HheC: 65 U mL^{-1}, HheB: 252 U mL^{-1}) in 500 μL of Tris-SO_4 buffer (200 mM, pH 7.5, 160 mM NaN_3) were stirred (120 rpm) at 30 °C for 24 h. Cyanide reactions: 27–35 μmol substrate, 10 mg rehydrated cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A or 20 μL of LBADH stock solution (5 mg mL^{-1}, 1 mg mL^{-1} NADPH) and 5 μL 2-propanol in 500 μL of Tris-SO_4 buffer (200 mM, pH 7.5, 160 mM NaN_3) were stirred (120 rpm) at 30 °C for 4 h, NaCN (4 mg) and 30–50 μL Hhe-preparation (HheC: 65 U mL^{-1}, HheB: 252 U mL^{-1}) were added and stirring continued for the appropriate time (see Experimental Section for further information). [b] Determined by GC analysis. [c] Determined by GC or HPLC employing a chiral stationary phase. [d] 70% of regioisomeric product (2-azido-2-phenylethanol) formed. [e] Not determined due to low conversion. [f] 41% of regioisomeric product (2-azido-2-phenylethanol) formed. [g] 10% of regioisomeric product (3-hydroxy-2-phenylpropanenitrile) formed. [h] 6% of regioisomeric product (3-hydroxy-2-phenylpropanenitrile) formed. [i] For a related approach see the literature.[19]

Finally, five of the cascade reactions employing ADH-A and HheC were performed on a 50-mg scale (Table 3). The products were isolated in reasonable yields and perfect optical purity (>99% *ee*).

Table 3. Cascade processes for the synthesis of β-azidoalcohols and β-hydroxynitriles[a] on a 50 mg scale.[a]

Entry	Product	GC yield [%]	Isol. yield [%]	*ee* [%][b]	α_D[20]
1	**4a**	86	61	>99 (*S*)	–6.0
2	**4b**	89	41	>99 (*R*)	+17.8
3	**4d**	83	56	>99 (*R*)	–5.5
4	**5a**	87	38	>99 (*S*)	–4.2
5	**5c**	93	53	>99 (*S*)	–53.2

[a] See Experimental Section for reaction conditions. [b] Determined by GC or HPLC employing a chiral stationary phase.

Conclusions

A biocatalytic cascade reaction system involving a three-step, two-enzyme sequence was successfully implemented. Prochiral α-chloroketones were transformed into optically pure β-azidoalcohols and β-hydroxynitriles, respectively. The stereoselectivity of the process depended exclusively on the alcohol dehydrogenase used in the first step; thus, by switching ADH each enantiomer was accessible in excellent optical purity. This method has proven to be applicable for a variety of substrates. ADH inactivation by cyanide could be overcome by slightly changing the process design and by adding the nucleophile after all of the chloroketone substrate was reduced.

Experimental Section

General: NMR spectra were recorded in $CDCl_3$ at 360 (^{1}H) and 90 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ = 0.00 ppm) and coupling constants (*J*) are given in Hz. TLC plates were run on silica gel 60 F_{254} and compounds were visualised either by spraying with Mo reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (100 g L^{-1}), $Ce(SO_4)_2\cdot 4H_2O$ (4 g L^{-1}) in H_2SO_4 (10%)] or by UV light. Optical rotation values ($[\alpha]_D^{20}$) were measured at 589 nm (Na line) in a 1-dm cuvette.

For anhydrous reactions, flasks were dried and flushed with dry argon just before use. Standard syringe techniques were applied to transfer dry solvents and reagents in an inert atmosphere of dry argon. Anhydrous THF was distilled from sodium under a nitrogen atmosphere. Petroleum ether (b.p. 60–90 °C) and EtOAc used for chromatography were distilled prior to use.

Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A were prepared as described previously.[14b] LBADH (#05.11, 500 U mL^{-1}, alcohol dehydrogenase from *Lactobacillus brevis*) was purchased from Codexis Inc. Redwood, USA, and Julich Chiral Solutions, a Codexis company, Germany. HheB (#45.30,

252 U mL^{-1}, halohydrin dehalogenase from *Mycobacterium* sp. GP1) and HheC (#46.30, 65 U mL^{-1}, halohydrin dehalogenase from *Agrobacterium radiobacter* AD1) were provided by Julich Chiral Solutions, a Codexis company.

Methyl-4-chloroacetoacetate (**1b**), 2-chloroacetophenone (**1c**), *rac*-1,2-epoxy-3-phenoxypropane (**3a**), *rac*-styrene oxide (**3c**) and *rac*-1,2-epoxyoctane (**3d**) were commercially available and used without further purification.

Synthesis of Substrates and Reference Compounds: Substrates 1-chloro-3-phenoxy-2-propanone (**1a**)[20] and 1-chloro-2-octanone (**1d**)[21] as well as reference compounds *rac*-1-chloro-3-phenoxy-2-propanol (**2a**),[22] *rac*-1-chloro-2-octanol (**2d**),[22] *rac*-methyl-3,4-epoxybutyrate (**3b**),[23] *rac*-1-azido-3-phenoxy-2-propanol (**4a**),[24] *rac*-2-azido-1-phenylethanol (**4c**),[6a] *rac*-2-azido-2-phenylethanol,[6a] *rac*-3-hydroxy-3-phenylpropanenitrile (**5c**)[25] and *rac*-3-hydroxy-2-phenylpropanenitrile[26] were synthesised as described in the literature. *rac*-Methyl-4-chloro-3-hydroxybutyrate (**2b**) and *rac*-2-chloro-1-phenylethanol (**2c**) were synthesised by $NaBH_4$ reduction of the corresponding ketones following a procedure given in the literature.[27] *rac*-Methyl-4-azido-3-hydroxybutyrate (**4b**) and *rac*-1-azido-2-octanol (**4d**) were synthesised by $CeCl_3$-mediated ring opening of the corresponding epoxides with NaN_3 following a procedure given in the literature.[24] Replacing NaN_3 with NaCN in the same procedure turned out to be a suitable method for the synthesis of the hydroxynitriles *rac*-3-hydroxy-4-phenoxybutanenitrile (**5a**), *rac*-methyl-4-cyano-3-hydroxybutyrate (**5b**) and *rac*-3-hydroxynonanenitrile (**5d**); a general procedure is given below. Synthesis of all compounds is fully described in the Supporting Information.

General Procedure for the Ring-Opening of Epoxides with NaCN Mediated by Cerium Chloride: To a solution of the epoxide (1.0 mmol) in MeCN/H_2O (9:1, 10 mL) was added NaCN (65 mg, 1.32 mmol) and $CeCl_3 \cdot 7H_2O$ (200 mg, 0.54 mmol). The suspension was heated at reflux for 24 h, cooled to room temperature and extracted with EtOAc (3 × 6 mL). The combined organic phases were washed with water (30 mL) and brine (30 mL), dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. Flash chromatography (silica; petroleum ether/EtOAc, 4:1) furnished the pure hydroxynitrile.

***rac*-3-Hydroxy-4-phenoxybutanenitrile (5a):** 157 mg (84%) of a yellowish oil. For characterisation see the Supporting Information.

***rac*-Methyl-4-cyano-3-hydroxybutyrate (5b):** 48 mg (35%; containing **2b** as residual impurity) of a yellowish oil. For characterisation see the Supporting Information.

***rac*-3-Hydroxynonanenitrile (5d):** 147 mg (92%) of a yellowish oil. For characterisation see the Supporting Information.

Epoxide Ring Closure and Ring Opening Catalysed by HheC Employing Different Concentrations of the Nucleophile: Tris-SO_4 buffers (200 mM, pH 7.5) bearing 8–640 mM of either NaN_3 or NaCN were prepared. Substrate **2a** (5 mg, 27 μmol) and HheC preparation (15 μL, 1.0 U) were dissolved in the Tris-SO_4 buffer (500 μL) and incubated for 4 h at 30 °C and 120 rpm. The samples were extracted with EtOAc (600 μL) and dried with Na_2SO_4. The conversion was determined by GC analysis.

Determination of the Influence of NaN_3 and NaCN on the Activity of ADH-A: Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A (5 mg) were rehydrated in Tris-SO_4 buffer (500 μL, 200 mM, pH 7.5, containing 160 mM NaN_3 or NaCN or none) for 1 h at 30 °C and 120 rpm. 2-Propanol (5 μL, 65 μmol) and **1b** (3 μL, 26 μmol) were added. The samples were incubated for 4 h at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried with Na_2SO_4. The conversion was determined by GC analysis.

General Procedure for the Three-Step Cascade Employing ADH-A, HheC and NaN_3: Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A (5 mg) were rehydrated in Tris-SO_4 buffer (500 μL; 200 mM, pH 7.5, 160 mM NaN_3) for 1 h at 30 °C and 120 rpm. 2-Propanol (5 μL, 65 μmol), HheC preparation (30 μL, 2.0 U) and the substrate (**1a**: 4 μL, 27 μmol; **1b**: 4 μL, 35 μmol; **1c**: 5.4 mg, 35 μmol; **1d**: 5 μL, 31 μmol) were added. The samples were incubated for 24 h at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried with Na_2SO_4. The conversion was determined by GC analysis.

General Procedure for the Three-Step Cascade Employing LBADH, HheB and NaN_3: A stock solution of LBADH (100 μL, 50 U) in Tris-SO_4 buffer (200 mM, pH 7.5, 1 mg mL^{-1} NADPH) was prepared. LBADH stock solution (10 μL), 2-propanol (5 μL, 65 μmol), HheB preparation (30 μL, 7.6 U) and the substrate (**1a**: 4 μL, 27 μmol; **1b**: 4 μL, 35 μmol; **1c**: 5.4 mg, 35 μmol; **1d**: 5 μL, 31 μmol) were added to Tris-SO_4 buffer (500 μL, 200 mM, pH 7.5, 160 mM NaN_3). The samples were incubated for 24 h at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried with Na_2SO_4. The conversion was determined by GC analysis.

General Procedure for the Three-Step Cascade Employing ADH-A, HheC and NaCN: Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A (10 mg) were rehydrated in Tris-SO_4 buffer (500 μL, 200 mM, pH 7.5) for 1 h at 30 °C and 120 rpm. 2-Propanol (5 μL, 65 μmol) and the substrate (**1a**: 4 μL, 27 μmol; **1b**: 4 μL, 35 μmol; **1c**: 5.4 mg, 35 μmol; **1d**: 5 μL, 31 μmol) were added, and the samples were incubated for 4 h at 30 °C and 120 rpm. HheC preparation (**a**, **d**: 30 μL, 2.0 U; **b**, **c**: 50 μL, 3.3 U) and NaCN (4 mg, 82 μmol) were added and incubation continued for 44 h. The samples were extracted with EtOAc (600 μL) and dried with Na_2SO_4. The conversion was determined by GC analysis.

General Procedure for the Three-Step Cascade Employing LBADH, HheB and NaCN: A stock solution of LBADH (100 μL, 50 U) in Tris-SO_4 buffer (200 mM, pH 7.5, 1 mg mL^{-1} NADPH) was prepared. LBADH stock solution (20 μL), 2-propanol (5 μL, 65 μmol) and the substrate (**1a**: 4 μL, 27 μmol; **1b**: 4 μL, 35 μmol; **1c**: 5.4 mg, 35 μmol; **1d**: 5 μL, 31 μmol) were added to Tris-SO_4 buffer (500 μL, 200 mM, pH 7.5, 160 mM NaN_3), and the samples were incubated for 6 h at 30 °C and 120 rpm. HheB preparation (50 μL; 7.6 U) and NaCN (4 mg, 82 μmol) were added and incubation continued for 18 h. The samples were extracted with EtOAc (600 μL) and dried with Na_2SO_4. The conversion was determined by GC analysis.

General Procedure for the 50 mg Scale Three-Step Cascade Employing ADH-A, HheC and NaN_3: Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A (50 mg) were rehydrated in Tris-SO_4 buffer (5 mL, 200 mM, pH 7.5, 160 mM NaN_3) for 1 h at 30 °C and 120 rpm. 2-Propanol (50 μL, 0.65 mmol), HheC preparation (300 μL, 20 U) and the substrate (**1a**: 40 μL, 0.27 mmol; **1b**: 40 μL, 0.35 mmol; **1d**: 50 μL, 0.31 mmol) were added. The mixture was incubated for 24 h at 30 °C and 120 rpm, extracted with EtOAc (2 × 10 mL), dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. Flash chromatography (silica; petroleum ether/EtOAc, 5:1) furnished the pure azidoalcohol.

(*S*)-1-Azido-3-phenoxy-2-propanol (4a): 32 mg (61%) of a yellowish oil. $[\alpha]_D^{20}$ = –6.0 (*c* = 1, $CHCl_3$), ref.[28] $[\alpha]_D^{20}$ = +19.63 [*c* = 2.5, $CHCl_3$; 30% *ee* (*R*)]. For characterisation see the Supporting Information.

(*R*)-Methyl-4-azido-3-hydroxybutyrate (4b): 23 mg (41%) of a yellowish oil. >99% *ee* (*R*). $[\alpha]_D^{20}$ = +17.8 (*c* = 1, $CHCl_3$), ref.[29] $[\alpha]_D^{20}$

= +19.63 [c = 1.03, $CHCl_3$; >99%*ee* (*R*)]. For characterisation see the Supporting Information.

(*R*)-1-Azido-2-octanol (4d): 30 mg (57%) of a yellowish oil. $[\alpha]_D^{20}$ = –5.5 [c = 1, $CHCl_3$; (*R*)], ref.[6a] $[\alpha]_D^{20}$ = –1.0 (c = 2.0, CH_2Cl_2; 30%*ee*). For characterisation see the Supporting Information.

General Procedure for the 50 mg Scale Three-Step Cascade Employing ADH-A, HheC and NaCN: Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-ADH-A (100 mg) were rehydrated in Tris-SO_4 buffer (5 mL, 200 mM, pH 7.5, 160 mM NaN_3) for 1 h at 30 °C and 120 rpm. 2-Propanol (50 μL, 0.65 mmol) and the substrate (**1a**: 40 μL, 0.27 mmol; **1c**: 54 mg, 0.35 mmol) were added, and the mixture was incubated for 4 h at 30 °C and 120 rpm. HheC preparation (500 μL, 20 U) and NaCN (40 mg, 0.82 mmol) were added and incubation continued for 44 h. The mixture was extracted with EtOAc (2 × 10 mL), dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. Flash chromatography (silica; petroleum ether/EtOAc, 5:1) furnished the pure hydroxynitrile.

(*S*)-3-Hydroxy-4-phenoxybutanenitrile (5a): 18 mg (38%) of a yellowish oil. $[\alpha]_D^{20}$ = –4.2 (c = 1, $CHCl_3$), ref.[30] $[\alpha]_D^{20}$ = –1.93 [c = 0.89, $CHCl_3$; 29%*ee* (*S*)]. For characterisation see the Supporting Information.

(*S*)-3-Hydroxy-3-phenylpropanenitrile (5c): 29 mg (53%) of a yellowish oil. $[\alpha]_D^{20}$ = –53.2 (c = 1, $CHCl_3$), ref.[31] $[\alpha]_D^{20}$ = +59.9 [c = 1.0, $CHCl_3$; >99%*ee* (*R*)]. For characterisation see the Supporting Information.

Supporting Information (see footnote on the first page of this article): Synthetic procedures and NMR spectroscopic data for all investigated compounds; conditions for GC, HPLC and GC–MS analysis; substance characterisation data; ^1H NMR spectra of reference compounds **2b** and **2c**, as well as ^{13}C NMR spectra of reference compounds **4b**, **4d**, **5a**, **5b** and **5d**.

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